

Masatoshi SAIKAWA\*: **Ultrastructure of septa of  
two species of Dimargaritaceae (Mucorales)**

犀川政稔\*: Dimargaritaceae (ケカビ目) 2 種の隔壁微細構造

(Plates IV—VI)

Under the light microscope, the fungi belonging to the Dimargaritaceae and the Kickxellaceae are observed to have characteristic cross wall structure with central perforation occluded by a biconvex plug (Benjamin 1959). On the other hand, Young (1969) and Benny & Aldrich (1975) showed the ultrastructures of septa in *Linderina pennispora* Raper et Fennell in the Kickxellaceae, in which bifurcated cross wall was clearly seen at its central perforation. It was highly developed and approached that of the dolipore septum in certain Basidiomycetes (Young 1969).

In the light microscopic observation, the septum in the hyphae of the Dimargaritaceae had, in addition, a bead-like protuberance at each pole of the biconvex plug. The generic name, *Dimargaris*, is derived from the presence of this structure (van Tieghem 1875). The purpose of this paper is to show the septum ultrastructure in two species of the Dimargaritaceae, *Dimargaris verticillata* Benjamin and *Dispira cornuta* van Tieghem. Up to now, there is no report concerning the electron microscopic observation of these fungi.

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**Materials and Methods** Cultures of *Dimargaris verticillata* (MIKAWA-110) and *Dispira cornuta* (MIKAWA-120) were presented by Dr. T. Mikawa of Tokyo Kyoiku University and were grown on YpSs agar plates at 20°C for 10 days with a host fungus, *Cokeromyces recurvatus* Poitras (IFO-6737).

\* Department of Biology, Tokyo Gakugei University, Koganei, Tokyo, 184. 東京学芸大学生物学教室.

The preparation procedure for electron microscopy is as follows. The mycelium grown on the agar plate was fixed with iced 5% glutaraldehyde in 0.07 M phosphate buffer (pH 7.2) for one hour and postfixed with 1%  $\text{OsO}_4$  in the same buffer overnight. An appropriate portion of the mycelium was then covered with a polyethylene film (5 mm  $\times$  5 mm  $\times$  0.03 mm) and cut off as an agar block. The block was coated with agar entirely, dehydrated with an ethanol series, and embedded with epoxy resin in a polyethylene dish. After polymerization of the resin, the casting was cut with a fret saw along the ridges of the film, whereupon the polymerized block was easily separated into two segments at the border of the polyethylene film. The material was thus found right below the subject surface of the resin. The ultrathin sections were cut with glass knives on a Porter Blum MT-2 ultramicrotome, poststained with aqueous uranyl acetate followed by aqueous lead citrate, and examined with a JEM-7 electron microscope.

**Observation** Longitudinal thin section through central part of the septum of a sporophore in *Dimargaris verticillata* clearly exhibits that the cross wall bifurcates at central part to form a lenticular cavity which is plugged by homogenous electron dense material (Plate IV). A bead-like protuberance is located at each pole of the biconvex plug. In the cytoplasm, a nucleus, several mitochondria and various sized vacuoles are also present.

Plate Va is the photographic enlargement of a part of Plate IV. One of the bead-like protuberances locating above the cross wall measured ca. 2  $\mu\text{m}$  in diameter. It is remarkable that the bead-like protuberance has less degree of the electron density.

Plate Vb shows a terminal end of a fertile hypha in *D. verticillata* with two sporiferous branchlets. The proximal part of one of the sporiferous branchlets has the typical septum apparatus which is also shown more clearly in Plate Vc by the photographic enlargement. Although the section is cut through median part of the septum, it is slightly oblique to the long axis of the sporiferous branchlet. In the lenticular cavity there is a dense material of the plug. The bead-like protuberances are seen at both side of the septum. Amorphous and highly electron-dense materials are located between the bead-like protuberance (left) and the plug.

Plate VIa is the cross wall at the branching part of the fertile hypha in *Dispira cornuta*. The cross wall structure is very similar to that of *Di-*

*margaris verticillata*. The well bifurcated cross wall, characteristic bead-like protuberances and the dense septal plug are observed. One of the two bead-like protuberances is slender in shape protruding from the lenticular cavity to the cytoplasm (right-hand side in Plate VIa). Electron-dense particles in the bead-like protuberance are very small and of various size. The septum apparatus in Plate VIb also shows the typical cross wall structure of fertile branches of *Dispira cornuta*. The dense particles in the bead-like protuberance are larger in size compared to those in Plate VIa. The cytoplasm is highly vacuolated.

**Discussion** The ultrastructure of septum apparatus in two species of the Dimargaritaceae was very similar to that in *Linderina pennispora* of the Kickxellaceae (Young 1969, Benny & Aldrich 1975). The cross wall was bifurcate at central perforation to form lenticular cavity in which the electron dense material was deposited as a plug (Benny & Aldrich 1975). Benny (1972) proved by histochemical study that the plug of four species of the Dimargaritaceae and three species of the Kickxellaceae was composed of lipids.

The bead-like protuberance of the septum apparatus is characteristic of the Dimargaritaceae. In the electron micrographs, it is homogenous in appearance like that of the plug. However, it is usually less dense than in the plug and contains electron-dense materials as tiny particles or larger amorphous deposits. From the electron micrographs, the bead-like protuberance of the Dimargaritaceae seems to be composed of lipids and is thought to be derived from the plug forming the dense particles.

Except the presence of the bead-like protuberance, the septum ultrastructure of the present two species of the Dimargaritaceae resembles that of the Trichomycetes which was already observed by other authors (Farr & Lichtwardt 1967, Lichtwardt 1973, Manier 1973, Moss 1975). However, bifurcation of cross wall in the Trichomycetes was less developed than in *Linderina pennispora* (Young 1969) and two species of the Dimargaritaceae in the present observation. In *Genisterospora homothalica* (Harpellales), the septal plug had remnants of the endoplasmic reticulum (Moss 1975), but in the two species of the Dimargaritaceae such a feature was not observed. Moss (1975) postulated the phylogenetical relationship between the Kickxellaceae and two orders (Harpellales and Asellariales) of the Trichomycetes.

By the present electron microscopic observation, the two species of the Dimargaritaceae must be added to the group because of the presence of this particular type of septum. Krisel (1969) classified the Dimargaritaceae and the Kickxellaceae under the order Kickxellales. In this connection, further observations of septum ultrastructure in the related species are also required.

### References

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### Explanation of Plates IV-VI

Plate IV. Longitudinal thin section of a sporophore of *Dimargaris verticillata*.

M: mitochondrion, N: nucleus, PL: septal plug, PR: bead-like protuberance, V: vacuole.

Plate V. *Dimargaris verticillata*. a: high magnification of a sporophore. b: terminal end of the fertile hypha, showing two sporiferous branchlets. c: high magnification of the proximal part of one of the sporiferous branchlets. Symbols as in Plate IV.

Plate VI. *Dispira cornuta*. a: septum apparatus of the young hypha. b: septum apparatus of the aged fertile hypha. Symbols as in Plate IV.

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ケカビ目 Dimargaritaceae に属する *Dimargaris verticillata* と *Dispira cornuta* の菌糸の隔壁を電子顕微鏡で観察した。菌糸の縦断切片をみると隔壁は中央の穴のところで 2 又に分かれており、レンズ形の空所を形成していた。その空所には常に高電子密度の物質がつまっており、栓となっていた。今まで、これとよく似た微細構造は接合菌綱のケカビ目 Kickxellaceae に属する *Linderina pennisporea* とトリコミケーテス綱の Harpellales と Asellariales の数種で報告されている。さらに、Dimargaritaceae の菌糸には隔壁の両側に球形の突出物があり、光学顕微鏡および電子顕微鏡の何れでも観察される。これはこの科だけの特徴である。